Focus Stacking for

Teaching and Publication in

Plant Sciences

Primary contact for the team

Jennifer Deegan, Department of Plant Sciences, University of Cambridge, jic24@cam.ac.uk

Team

Jennifer Deegan, jic24@cam.ac.uk

Visitor, Department of Plant Sciences, University of Cambridge.

Designing, building and operating the setup. Writing the curriculum for knowledge transfer.

Richard Mortier, richard.mortier@cl.cam.ac.uk

Department of Computer Science and Technology, University of Cambridge. Sponsor for the research and cost centre.

Tim Deegan, tjd@phlegethon.org

External collaborator in the computing industry. Technical advisor in home robotics, computer programming, electronics and optics.

Christopher Whitewoods, Christopher.Whitewoods@jic.ac.uk

Department of Cell and Developmental Biology, John Innes Centre, Norwich.

Chris works on trap formation in the carnivorous plant *Utricularia gibba*, and is interested in the cellular basis of organ shape. He will provide plant material for Jennifer to grow and photograph.

Matthew Couchman, matthew.couchman@jic.ac.uk

Computational and Systems Biology, John Innes Centre, Norwich.

Matthew Couchman is helping set up further collaborations.

Aleksandr Gavrin, Aleksandr.Gavrin@slcu.cam.ac.uk

Sainsbury Laboratory, University of Cambridge

Alex works on mutant trichomes in *Arabidopsis* and so needs deep focus images of the trichomes at high magnification. He will provide photographic specimens for use with the microscope system.

Summary

Within the 6 month period of the grant, our aim is to design a £100 teaching tool and a curriculum to teach focus stacked photography to secondary school children and undergraduates.

We will also extend our DIY professional-grade system so that it can photograph subjects from 0.25mm to 1cm and beyond. The system’s current range is 0.5mm to 2.5mm (See attached photos). This will enable capture of unique plant science images and will be a valuable teaching tool in its own right. This system will be fully documented so that it can be adapted and copied by other groups.

Within and beyond the period of the grant, our aim is to use the system to take deep focus images of *Arabidopsis* trichomes, *Utricularia gibba* traps, and developing fern gametophytes.

We hope that making these images available will start a trend for sharing of microscopic deep focus plant science images, and provide tools for school and University teaching staff to ignite enthusiasm for these tiny plant specimens.

Proposal

It has only recently become possible to take photographs of tiny plant specimens and have the full subject in focus. The technology is called focus stacking or deep focus photography. In this technique a large number of photographs are taken, while gradually moving the camera towards the subject. Each photograph has only some small areas in focus, and these focused areas are then cut out and merged together to make one, completely focused, image.

This technology is important for phenotyping in synthetic biology using *Marchantia*. It is also needed in very many other areas of biology research where the subject is very tiny. For example, in trichome research in *Arabidopsis*.

Aim

To advance the use of deep focus photography in two ways:

1. Develop a £100 teaching tool that can be used to teach the principles of deep focus photography, and a curriculum to accompany it.

2. Demonstrate and document how to convert the system so that it can take publishable photographs of the sort that can appear on the front cover of a journal, to be built for under £4000. Our system will accommodate subjects from 0.25mm to 1cm tall and beyond.

Method

We are building on our already successful focus stacking system, which won the Biomaker Challenge 2017 Competition (https://tinyurl.com/y89ysqyu). The system provides a very suitable base for the work. It was built cheaply, and so is easily adapted to this new teaching purpose.

We have a full understanding of how our system works, having built it ourselves, right down to the electronics and software that drives the rail. This knowledge and experience will help very much in writing the curriculum to teach others, and in designing a tool to help them to learn. The most critical part of our system is the rail on which the camera is mounted, and moved slowly towards the subject. Our rail is built from a focus block cut out of an old microscope. We know that this is a relatively easy thing for a school or University department to find, and a good base for an inexpensive teaching tool.

Who will be involved?

Our team is highly interdisciplinary, and brings together expertise in programming, computer hardware, optics, photography, and plant science. We have found two new collaborators in Cambridge and at the John Innes Centre who need our technology to photograph their biological specimens (*Arabidopsis* trichomes and *Utricularia gibba* traps). The team members and their contributions are listed in the “Team” section, above and in the “Outcomes” section, below.

Outcomes

First six months

1. Our £100 teaching tool and accompanying curriculum will be suitable for teaching the principles of deep focus photography at secondary school and University level. We anticipate that it will be widely copied and provide a good general introduction to focus stacking, and a detailed training for young biologists coming through.

2. Our professional-grade system will demonstrate how to build a setup to take publishable photographs for under £4000. This price compares very favourably with commercial systems such as the Keyence microscope, which starts at £54,000. Our project will enable every GMO and synthetic biology lab to build their own set of equipment, rather than needing to move their specimens to a different institute to be photographed on shared commercial equipment.

Within or beyond the first six months

In the longer term, our system will be used to take publishable photographs of significant experimental plant species and parts. We would like to start with the following:

1. A developmental series of the fern gametophyte from filamentous stage, to the emergence of the first sporophyte leaf. (Jennifer Deegan)

2. Mutant and wild type *Arabidopsis* trichomes (Aleksandr Gavrin).

3. *Utricularia gibba* trap formation in the carnivorous plant *Utricularia gibba*, and the cellular basis of organ shape (Christopher Whitewoods).

We hope to use these photographs to create educational displays to exhibit at RHS exhibitions in London, and to distribute for use in text books and biology classes at school and University level.

With a professional-grade system working at a full range of magnifications, we will be able to photograph a whole range of plant structures, that have never before been shown in text books and teaching materials.

This will be a novel, publishable contribution to plant science research, and a significant resource in training and knowledge transfer at secondary school and University level.

Benefits and outcomes

How does our project fit the remit of OpenPlant and the judging criteria?

Synthetic biology

Deep focus photography is a big feature in synthetic biology, where it is being used for phenotyping (e.g. in the Haseloff lab in Cambridge). Our low-cost teaching tool will enable this technology to be taught easily to undergraduates in synthetic biology classes. Our professional system will also be more affordable for lab groups than the commercial systems, which start at around £54,000. This will enable synthetic biology groups to have their own inexpensive phenotyping equipment in each lab.

Collaboration between the John Innes Centre and Cambridge

Jennifer Deegan is carrying out most of the work, and is based in Cambridge, and she has established two new collaborations with postdocs in Norwich and Cambridge. One is with Chris Whitewoods who is based at the John Innes Centre and studies traps in the carnivorous plant *Utricularia gibba*. The second is with Aleksandr Gavrin, who studies trichome mutants in *Arabidopsis*. He works at the Sainsbury Laboratory in Cambridge. We anticipate that from these new alliances, further collaborations will arise.

Open technology and responsible innovation

Our initial system has been documented in great detail on Hackster (https://tinyurl.com/y89ysqyu), and we will continue in the same way in this project, if that is the preferred system of the organisers. This will make our design accessible world-wide for anyone who wishes to copy it.

Our teaching curriculum and tool will actively encourage others to copy our work. We are happy for anyone to do this, and ask only that they should cite our publication of the build methods (currently Deegan, J., and Deegan, T., The Pteridologist Magazine 2018).

We will seek the guidance of the judges on which one format they would like the curriculum to be presented in. Formats that we might suggest and can accommodate include:

- Printable pdfs
- A website
- Blog style
- Hackster tutorial
- As a published article

We would suggest that the platform chosen should be one that will persist and can be converted easily, as file formats change. For example pdfs on an open access University server, or in a special edition of an open access journal that will be archived online. These could be linked from Hackster.

We will include our citation and the creative commons licensing terms on each page. This way, the users will know that they are welcome to use, share and build on our work.

Together, this will ensure that the guides are accessible for use by all, in line with the open technology commitment of the OpenPlant inititative.

Interdisciplinary team

Our team is highly interdisciplinary, bringing together two academic biologists (Christopher Whitewoods and Aleksandr Gavrin), a computer scientist (Tim Deegan) and a further team member with experience in both areas (Jennifer Deegan).

- Chris and Alex are both in academic plant science research and are able to use the system directly on their own experimental plant subjects.

- Tim Deegan is a computer scientist working in industry, and is helping as a volunteer with the details of the electronics, computer programming, optics and engineering. His skills are expensive and hard to come by, and this project could not be carried out without his help.

- Jennifer Deegan is on career break raising her son and is driving this project and doing most of the work, also as a volunteer. She previously had a career in plant science and bioinformatics, so can work with both communities. Jennifer has particular strengths in writing teaching materials for biological computing subjects. She previously spent 7 years as a computer programmer, and as the outreach and training specialist for the Gene Ontology Consortium, working from the EMBL-European Bioinformatics Institute. Her previous career was as a plant developmental biologist at the John Innes Centre and at Cambridge University, so she is well placed to work with all members of the team, and to complete the project.

Tangible contributions to training and knowledge exchange

1. A low-cost teaching tool and a full curriculum for use of the tool. This will include worksheets and the technical explanations suitable for use at secondary school and University level.

   All documentation will be placed online for free use by anyone, under a suitable creative commons licence, probably creative commons CC BY 4.0.

2. A low cost professional-grade photography system that can be used to illustrate text books, journal articles and teaching materials.

   Build-instructions will be placed online for free use by anyone, under a suitable creative commons licence, probably creative commons CC BY 4.0.

3. By introducing this new form of photography to plant science we hope to start a trend for sharing of microscopic images that has not previously been possible.

   Ideally, we aim to have the images on the front covers of journals in which the work on the plant material has been published. (As in our previous work using a different setup, see attached image cover.pdf).

   We could also encourage sharing of images on twitter with a new hashtag like #DeepFocusBotany. The hashtag could perhaps be started in Jennifer Deegan's personal twitter account and then taken up by the Cambridge University Department of Plant Sciences, and John Innes Centre twitter accounts if it seems to be working well.

4. We would like our images to be made accessible online to be used by textbooks, academic societies, secondary schools and Universities worldwide.

   Licensing of photographs is in flux and is a tricky area. There is not currently a suitable creative commons licence for controlling use of digital photographs. The freedom to copy that these licences offer currently leads to images being used inappropriately, losing attribution, and occasionally being attributed to other photographers or institutes in error.

   In the absence of a universally suitable CC license we would like to propose the following:

   - Small size versions of the images will be offered free online for use in teaching and training purposes, on a creative commons licence (probably the CC BY 4.0 license). This will allow anyone to use the small images in any way.

   - Full sized copies of the images will be made available free for academic uses, on a case-by-case basis. For example this could be as publicity for the Pteridological Society, the Department of Plant Sciences in Cambridge, and the John Innes Centre.

   - Commercial uses of full-sized images may incur a license fee, and will be considered on a case-by-case basis under traditional Rights Managed Licensing. This ensures that the photographer must be contacted before an image can be used, and that inappropriate uses can be prevented.

Realistic given the time, costing and team involved

We anticipate that these goals would be very realistic, given the time, costing and team involved. Most of the same team were involved in the Biomaker Challenge 2017 and we were successful in completing and fully documenting our project in well under the required amount of time.

We will be working on similar types of goals this time. This includes building a similar style of device, and writing a very large amount of documentation.

We have a clear idea of what the time constraints will be and what we will need to do to complete the work, and we are confident that we can finish within the allowed time limit.

Sponsor for the research and cost centre

Richard Mortier, richard.mortier@cl.cam.ac.uk

Department of Computer Science and Technology,

University of Cambridge.

I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

Building the setup

We will buy the following three items:

20X M Plan Apo SL (30.5mm WD) £1544
5X M Plan Apo Objective £632
2X M Plan Apo Objective £592.92

These prices both include VAT and educational discount.

Documentation

Writing documentation is the major part of this work and we would like to include a large number of illustrations. It would be helpful if we could use some of the money to buy or rent a second camera to take photos of our own photography setup. This is currently tricky as our camera is in the photos.

We would also like to ask the judges how they would like the documentation presented (web, print or both). We would then consider spending some of the grant money on buying or leasing software to speed up production of the documentation.

Other

We would like to leave some of the budget unaccounted for as we found a number of unexpected problems during the Biomaker Challenge 2017, and we expect the same to happen this time. It would help to ensure that we complete the project on time if we have some spare funds to solve problems.

We do not have other sources of funds for this equipment.

Matched funding

We have already found funding of several thousand pounds to develop our system. Most of this money came from personal savings of Jennifer Deegan, and there was also £1000 from the Biomaker Challenge. Two of our team members are giving their time as volunteers, which represents a substantial financial saving for the project.